PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2002.014 WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09031	International filing date *(day/month/year)* 12.08.2003	Priority date *(day/month/year)* 13.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/86		
Applicant AKZO NOBEL N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.03.2004	24.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Brouns, G Telephone No. +31 70 340-3789

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09031

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21 as originally filed

Claims, Numbers

1-12 filed with telefax on 21.12.2004

Drawings, Sheets

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	3,5
	No:	Claims	1, 2, 4, 6-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	-

2. Citations and explanations

   see separate sheet

The application discloses Bovine viral diarrhea virus (BVDV) replicons, with a deletion in either the capsid (C) protein or in the glycosylated envelope protein E1, whereby the parts of C and E1 that function as signal sequence for E^{ms} and E2, respectively, are retained to ensure proper expression of said structural proteins. Infectious particles have been produced by transfection of *in vitro* transcribed replicon RNA into cell lines that trans-complement the lacking C or E1 gene, and the resulting virus has been shown to be replication deficient. Replication deficient virus lacking functional C protein has been used in vaccine compositions.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) The amended claims filed with the letter of 21.12.2004 fulfil the requirements of Article 34(2)(b) PCT because said amendments do not go beyond the disclosure of the international application as filed.

2) Reference is made to the following documents:

D1: EP-A-1035205 (Stichting Dienst Landbouwkundig Onderzoek, Lelystad NL) 13 September 2000 (2000-09-13)

Novelty

3) The prior art does not disclose a replicon of a pestivirus which is incapable of expressing one or more structural proteins of the virus, characterized in that said replicon expresses all structural proteins of a pestivirus except for a functional C and/or E1 protein, but wherein the signal sequences of the C and/or E1 protein essential for further downstream processing are retained or replaced by a coding sequence encoding analogous signal sequences from another pestiviral virus.
Furthermore, no infectious particles of pestivirus comprising said replicon, no vaccine comprising said infectious viral particles and no method for the production of aforementioned infectious viral particles of pestivirus has been indicated in the prior art.
The subject-matter of claims 1-12 is therefore novel (Article 33(2) PCT).

Inventive step

4.1) Document D1, which is considered to represent the most relevant state of the art for claim 1, discloses (cf. D1 experiments 1-3) a replicon of a pestivirus incapable of expressing a functional E^{ms} or E2 structural protein. From this the subject-matter of claim 1 differs in that the replicon of a pestivirus is incapable of expressing a functional E1 structural protein, but retains the signal sequence required for further downstream processing of E2.

The problem to be solved by the present invention may therefore be regarded as the provision of an alternative pestivirus replicon.

The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D1 teaches that in addition to E^{ms} and E2, also other pestivirus structural genes, C and E1, are suitable candidate genes that may be deleted in a pestivirus replicon for generating replication deficient infectious pestiviral particles (D1, description, page 3). In particular it is suggested to delete amino acids 500-665 of the E1 protein of chronic swine fever virus or the corresponding region of E1 protein of other pestiviruses. Since this E1 deletion mutant is almost identical to the E1 deletion mutant disclosed in the present application, it is considered to be suitable to practise the present invention.

The person skilled in the art would therefore generate a replicon lacking a functional E1 protein without use of his inventive skill and with reasonable expectation of success. In conclusion, no inventive step has been acknowledged for the subject-matter of claim 1 as well as claims 2 and 6-8, which relate to similar subject-matter (Article 33(3) PCT).

4.2) The selection of another pestivirus, BVDV (claims 4, 10, 11), the generation of viral particles using said replicon and cells that provide the deleted protein *in trans* (claim 9), and use of said replication defective viral particles in a vaccine preparation (claim 12) are routine solutions for the skilled person, therefore claims 4 and 9-12 lack inventive step (Article 33(3) PCT).

4.3) The prior art does not suggest to generate a pestivirus replicon in which part of the C protein is deleted, whilst retaining the C-terminal portion of said protein which functions as signal sequence for the E^{ms}E1E2 polyprotein.
Therefore, an inventive step has been acknowledged for the subject-matter of claims 3 and 5, as well as claims 8-12 when restricted to replicons in which part of the C protein

is deleted (Article 33(3) PCT).

Additional remarks

5.2) Claim 1 refers to coding sequences 'essential for further downstream processing', which is a functional definition. It seems that said coding sequence can be defined more precisely without unduly restricting the scope of the claims by introduction of structural and technical features of the claimed coding sequence, for which a basis is found in the application as originally filed, for instance as defined on page 3, lines 28-29 of the description.

***

AMENDED CLAIMS:

1. A replicon of a pestivirus which is incapable of expressing one or more structural proteins of the virus, characterized in that said replicon expresses all structural proteins of a pestivirus except for a functional C and/or E1 protein, but wherein the coding sequences encoding the part of the C and/or the E1 protein essential for further downstream processing are retained or replaced by a coding sequence encoding analogous signal sequences from another pestiviral species.
2. A replicon according to claim 1, characterized in that at least part of the coding sequence of the E1 or C protein has been deleted from said replicon.
3. A replicon according to claim 1 or 2, characterized in that said replicon does not encode a functional C protein.
4. A replicon according to claim 1 or 2, characterized in that said replicon is of the Bovine Viral Diarrhea Virus (BVDV).
5. A replicon according to claim 4, characterized in that the coding region encoding amino acid positions 201-243 of the C protein have been deleted.
6. A replicon according to claim 1 or 2, characterized in that said replicon does not encode a functional E1 protein.
7. A replicon according to claim 4, characterized in that the coding region encoding amino acid positions 498 to 653 of the E1 protein have been deleted.
8. Infectious viral particle of Pestivirus, characterized in that it contains a replicon according to any of claims 1-7.
9. A method for the production of viral particles of a Pestivirus according to claim 8, characterized in that said method comprises the following steps:
   a. Providing cells that are permissive for the Pestivirus and express Pestiviral E1 and/or C protein,
   b. Transfecting said cells with in-vitro transcribed RNA of a replicon according to any of claims 1 to 7,
   c. Culturing transfected cells obtained in step b,
   d. Harvesting the viral particles from the cultured cells.
10. A method according to claim 9, characterized in that said pestivirus is BVDV.
11. A method according to claim 10 or 11, characterized in that said cells express the E1 and/or C protein of BVDV.
12. A vaccine containing infectious viral particles according to claim 8 and a pharmaceutically acceptable carrier.

intervet

Intervet International bv
Patent Department
Wim de Körverstraat 35
P.O. Box 31
5830 AA Boxmeer
The Netherlands

Traderegister 16028015